

Quantum dot-based diabetic foot mapping for diagnosing osteomyelitis and Charcot neuroarthropathy



Farzaneh Yousefi^a, Iraj Nabipour^a, Mohammadreza Kalantarhormozi^a, Tahereh Assadi^a, Alireza Raeisi^a, Majid Assadi^{b,*}

^a Department of Endocrinology, The Persian Gulf Tropical Medicine Research Center, Bushehr University of Medical Sciences, Bushehr, Iran

^b The Persian Gulf Nuclear Medicine Research Center, Bushehr University of Medical Sciences, Bushehr, Iran

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ABSTRACT

The location of osteomyelitis is very important in Charcot neuroarthropathy (CN), especially when a physician is considering amputation of the affected extremity.

In diabetic CN, the presence of osteomyelitis is likely. Thus, to identify the infected tissue that needs to be removed, the specific area of infection must be correctly identified. Both CN and osteomyelitis have high mortality rates, but osteomyelitis is more life threatening and needs aggressive treatment.

We propose a QD-based method for distinguishing CN with sterile inflammation from osteomyelitis that does not require multiple and frequent imaging modalities. The method utilizes two different colored QDs (i.e., red and green). The red QD is attached to a UBI, an antimicrobial peptide, which attaches to bacteria, enabling their detection. The green QD is attached to MDP, which accumulates in areas of inflammation. When these QDs are injected intravenously at the same time, the red QD-UBI accumulates in infected areas and attaches to bacteria, and the green QD-MDP accumulates both in areas with sterile inflammation and infected areas.

The accumulation of only green QDs in the suspect extremity signifies a sterile inflammation process (CN). However, the accumulation of both the red and green QDs signify infectious and inflammation processes (i.e., osteomyelitis or a soft tissue infection, depending on the location). In the latter case, the treatment needs to be more intensive, with even amputation considered.

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Introduction

Diabetes is one of the most common diseases in the world, and its prevalence is increasing rapidly. According to a report by the American Diabetes Association, the number of diabetic patients in the U.S. increased from 25.8 million in 2010 to 29.1 in 2012 [1]. Diabetes is not a curable disease, but it can be managed to prevent serious consequences, such as neuropathy, retinopathy, diabetic nephropathy, gastric paresis, cardiovascular disease, hypertension, and infections [2].

Osteomyelitis

Osteomyelitis of the foot in diabetic patients is largely a consequence of several diabetes-related complications, especially

neuropathy, and, to a lesser degree, vasculopathy and defects in immunity and wound healing [3]. In osteomyelitis, the infarcted part of the foot (usually the forefoot or a toe) is dull red and diffusely swollen and warm. It may also be discharging pus or fragments of bone. Systemic signs of disease, such as fever and malaise, are unusual with foot infections, including osteomyelitis [4–10]. A clinical examination may lead to a suspicion of osteomyelitis, but further evaluation is needed to confirm the disease. If bone can be felt with the tip of a sterile metal probe inserted in the wound (probe-to-bone test), then bone infection (defined histologically) is likely. The probe-to-bone test has a sensitivity of only 66%, but it is relatively specific (84%) and has a negative predictive value of 62%. Laboratory findings, such as leukocytosis, and tests of ESR and CRP, which are increased in osteomyelitis, are useful in the diagnosis. A study showed that ESR higher than 70 mm/h indicated bone infection, with 100% specificity and 50% sensitivity [5]. Imaging modalities are also helpful in the diagnosis of osteomyelitis. Plain X-rays have poor sensitivity in the early stages of the disease [6]. In contrast, ^{99m}Tc-diphosphonate can demonstrate abnormal uptake as long as 2 weeks before abnormalities are seen on plain radiographs

* Corresponding author at: The Persian Gulf Nuclear Medicine Research Center, Bushehr University of Medical Sciences, Bushehr 3631, Iran. Tel.: +98 771 2580169; fax: +98 771 2541828.

E-mail addresses: assadipoya@yahoo.com, asadi@bpums.ac.ir (M. Assadi).

[7]. As ^{111}In -labeled leukocytes are not incorporated into areas of active bone turnover, the accumulation of labeled leukocytes is relatively specific for infection, especially in cases where other osseous abnormalities are present [7]. Tc-UBI, a Tc-labeled cationic antimicrobial peptide derived from ubiquicidin, binds strongly to microorganisms [8]. Akhtar et al. showed that the sensitivity, specificity, and overall diagnostic accuracy of Tc-UBI 29–41 in infection localization were 100%, 80%, and 94%, respectively. The positive and negative predictive values of Tc-UBI 29–41 were 92.9% and 100%, respectively [9]. In previous study, the accuracy of a 99m Tc-UBI scan in the diagnosis of osteomyelitis was very high [9].

MRI has an important role in the diagnosis of osteomyelitis. Marrow edema associated with inflammation is responsible for the characteristic changes seen in MRI of osteomyelitis. The diagnostic sensitivity of MRI for osteomyelitis has generally been reported to be 90–100%. Its specificity is somewhat limited due to the difficulty in MRI distinguishing osteomyelitis from other causes of marrow edema, including acute neuropathic osteoarthropathy [10–12].

A bone biopsy in diabetic patients with suspected osteomyelitis can be done. However, it is an expensive procedure, requires experience and technical skills, and takes several days to process [13]. It may also yield false negative results, either because of patchy infectious involvement or because of previous antibiotic therapy [10].

Osteomyelitis is a difficult infection to treat, largely because of limited blood flow in bone. As relapsing or repeated infections are common in diabetic foot osteomyelitis, surgical resection of the infected bone is often necessary [3,14].

Diabetic Charcot neuroarthropathy

Charcot neuroarthropathy (CN) is a condition affecting the bones, joints, and soft tissues of the foot and ankle, and it is characterized by inflammation in the earliest phases [15]. The clinical presentation and imaging findings of CN are challenging, as they are very similar to those of osteomyelitis. Both CN and osteomyelitis can coexist in an extremity, making the diagnosis even harder [16–19].

The sensitivity and specificity (<50%) of plain radiography in detecting early-stage CN are low [20]. Technetium-99m methylene diphosphonate 99m (TC-MDP) is positive in all three stages of CN and reflects the increased turnover of bone [20,21]. 99m TC and ^{111}In -WBC do not accumulate at sites of new bone formation without infection [21]. MRI is the most sensitive modality in detecting early changes in CN, with 90–100% sensitivity and 40–100% specificity [21–25].

The treatment aim in CN is to arrest the acute process to prevent the development of permanent deformity and to relieve pain, mostly by conservative treatment [26–28].

Quantum dots (QDs)

Quantum dots (QDs), tiny light-emitting particles at the nanometer scale, are emerging as a new class of fluorescent probe for in vivo bimolecular and cellular imaging [29]. QDs are extremely uniform, possess a high surface to volume ratio, and are endowed with intrinsic fluorescent properties, including very bright intensity and photostability. Moreover, the intermittent fluorescence emission (optical blinking) and electron dense nature of QD nanoparticles allow for easy identification of individual

nanoparticles in cell preparations [30,31]. In vitro applications of QDs are bimolecular tracking in cells, cellular imaging, and tissue staining. In vivo applications of QDs are biodistribution [32], vascular imaging [33], QD tracking [34], and tumor imaging [35].

With the development of biomarkers in cell biology, the tracking of some specific cells (such as cancer cells) becomes possible. Gac et al. have successfully detected apoptotic cells by conjugating QDs with biotinylated Annexin V, which enables the functionalized QDs to bind to phosphatidylserine (PS) moieties present on the membrane of apoptotic cells but not on healthy or necrotic cells [36]. Liu et al. reported the development of Gd-doped ZnO QDs with enhanced yellow fluorescence, and these QDs can be used as nanoprobes for quick cell detection with very low toxicity [37]. In 2007, Bagalkot et al. reported a more complex QDs-aptamer- (Apt-) doxorubicin (Dox) conjugate system [QD-Apt(Dox)] to endow QDs with the capability of targeting, imaging, therapy, and sensing the prostate cancer cells that express the prostate-specific membrane antigen (PSMA) protein [38].

In addition to their usage as nanoprobes and labels for in vitro imaging, QDs have also been widely used as in vivo imaging agents. Cancer-specific antibody, coupled to near-IR QDs with polymer coatings is the most popular QDs agent for tumor targeted imaging [39]. One study used nude mice for in vivo imaging after near-IR QD800-labeled BcaCD885 cells (BcaCD885/QD800) being implanted. Fluorescence signals of QDs accumulated in the tumor could be detected after 16 days of incubation at certain concentrations. It suggested that, compared with CT and MRI, QD800-based imaging could efficiently increase the sensitivity of early diagnosis of cancer cells [40].

Hypothesis: QD-based method for distinguishing CN and osteomyelitis

Due to the similar clinical presentation of CN and osteomyelitis and their similar imaging findings, the diseases are not easily distinguishable, but they require different management and treatment.

The location of osteomyelitis is very important in CN, especially when a physician is considering amputation of the affected extremity.

In diabetic CN, the presence of osteomyelitis is likely. Thus, to identify the infected tissue that needs to be removed, the specific area of infection must be correctly identified. Both CN and osteomyelitis have high mortality rates, but osteomyelitis is more life threatening and needs aggressive treatment.

We propose a QD-based method for distinguishing CN with sterile inflammation from osteomyelitis that does not require multiple and frequent imaging modalities (Fig. 1). The method utilizes two different colored QDs (i.e., red and green). The red QD is attached to a UBI, an antimicrobial peptide, which attaches to bacteria, enabling their detection. The green QD is attached to MDP, which accumulates in areas of inflammation. When these QDs are injected intravenously at the same time, the red QD-UBI accumulates in infected areas and attaches to bacteria, and the green QD-MDP accumulates both in areas with sterile inflammation and infected areas.

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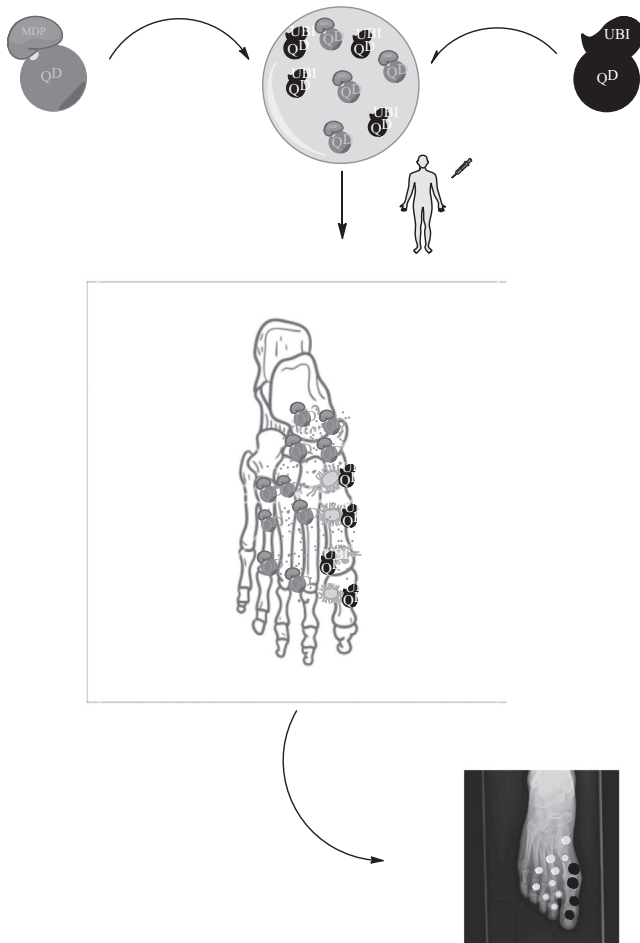


Fig. 1. QD-based method for distinguishing CN and osteomyelitis.

Disclosure statement

The authors have nothing to disclose.

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